

SCREENING OF FACTORS AFFECTING THE ENZYMATIC HYDROLYSIS OF FIBER PRESSED OIL PALM FROND (FPOPF)

NUR FATIN BINTI MUHAMMAD

Thesis submitted in partial fulfilment of the requirements
for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

**Faculty of Chemical & Natural Resources Engineering
UNIVERSITI MALAYSIA PAHANG**

JUNE 2015

©NUR FATIN BINTI MUHAMMAD (2015)

ABSTRACT

This paper reviews the alkaline pretreatment and factors that affecting the enzymatic hydrolysis oil palm frond (OPF). The factors studied in enzymatic hydrolysis are enzyme concentration, pH, temperature, reaction time and agitation speed. OPF is one type of agricultural wastes that consist of lignocellulosic material which comprised of cellulose, hemicellulose and lignin. Lignin is extremely strong component which resistant against chemical, enzymatic and bacteria breakdown. Thus, usage of lignocellulosic as a raw material involves alkaline pretreatment to be able to make the cellulose and hemicellulose fractions easily accessible for enzymatic hydrolysis. Dried OPF was pretreated by soaking into sodium hydroxide solution at 100°C for about 1 hour. For hydrolysis, the pretreated OPF was soaked into sodium acetate buffer and enzyme was applied too. The, the mixture was incubated in an incubator shaker. After that, the mixture was boiled in water bath for 10 minutes. The hydrolysates were quantified by using DNS method. After analyzed with Design Expert Software, pH gives the most contribution to the enzymatic hydrolysis. Lignocellulose material is known as a potential biomass for conversion into renewable sugar to produce value-added product such as biofuels, animal feeds, enzyme and healthy food products.

ABSTRAK

Kertas kerja ini mengkaji prarawatan alkali dan faktor-faktor yang memberi kesan kepada hidrolisis enzim pelepah kelapa sawit (OPF). Faktor-faktor yang dikaji dalam hidrolisis enzim adalah kepekatan enzim, pH, suhu, masa tindak balas dan kelajuan putaran. OPF adalah salah satu jenis bahan buangan pertanian yang terdiri daripada bahan lignoselulosa yang mengandungi selulosa, hemiselulosa dan lignin. Lignin adalah komponen yang amat kuat dan tahan terhadap kimia, enzim serta kerosakan bakteria. Oleh itu, penggunaan lignoselulosa sebagai bahan mentah melibatkan prarawatan alkali dapat membuat komponen selulosa dan hemiselulosa mudah diakses untuk hidrolisis enzim. OPF yang telah dikeringkan diprarawat dengan merendam ke dalam larutan natrium hidroksida pada suhu 100 ° C selama lebih kurang 1 jam. Untuk hidrolisis, OPF yang diprarawat telah direndam ke dalam buffer natrium asetat dan enzim telah diaplikasikan juga. Campuran tersebut telah dieram dalam inkubator. Selepas itu, campuran itu direbus dalam air selama 10 minit. The hidrolisat telah diukur dengan menggunakan kaedah DNS. Setelah dianalisis dengan Design Expert Software, pH memberikan sumbangan yang paling penting untuk hidrolisis enzim. Bahan lignoselulosa dikenali sebagai biomass yang berpotensi untuk dijadikan gula untuk menghasilkan produk seperti biofuel, makanan haiwan, enzim dan produk makanan yang sihat.

TABLE OF CONTENTS

SUPERVISOR’S DECLARATION	IV
STUDENT’S DECLARATION	V
<i>Dedication</i>	VI
ACKNOWLEDGEMENT	VII
ABSTRACT.....	VIII
ABSTRAK.....	IX
TABLE OF CONTENTS.....	X
LIST OF FIGURES	XII
LIST OF TABLES	XIII
LIST OF ABBREVIATIONS.....	XIV
LIST OF ABBREVIATIONS.....	XV
Chapter 1 Introduction	1
1.1 Background	1
1.2 Problem statement.....	1
1.3 Research objective	1
1.4 Research scope.....	2
1.5 Rationale and significance	2
1.6 Organization of thesis	2
Chapter 2 Literature Review	3
2.1 Lignocellulose Biomass	3
2.2 Oil Palm	4
2.3 Oil Palm Biomass	5
2.3.1 Oil Palm Frond.....	8
2.4 Lignocellulose component in Lignocellulose Biomass	10
2.4.1 Lignin.....	10
2.4.2 Hemicellulose	11
2.4.3 Cellulose	12
2.5 Application of Lignocellulose Component.....	13
2.5.1 Chemicals.....	15
2.5.2 Bio-fuel	15
2.5.3 Other high-value bioproducts	15
2.6 Availability of Lignocellulose Source (OPF)	16
2.6.1 Total oil palm plantation area	16
2.6.2 Frond availability from waste palm trees	18
2.7 Pretreatment	19
2.7.1 Physical treatment.....	20
2.7.2 Chemical treatment	21
2.7.3 Physicochemical treatment	22
2.8 Enzymatic hydrolysis.....	23
Chapter 3 Materials and Methodology	24
3.1 Feedstock collection and preparation	24
3.2 Enzyme	24
3.3 Alkaline pretreatment	24
3.4 Enzymatic hydrolysis.....	24

3.5 Reducing sugar analysis.....	24
3.6 Statistical analysis.....	25
Chapter 4 Result & Discussion.....	26
Chapter 5 Conclusion.....	33
References.....	34

LIST OF FIGURES

Figure 2.1: The first and second generations of lignocellulosic sources.....	3
Figure 2.2: The photograph of oil palm tree	5
Figure 2.3: WPT at 25 years of age consists of different physical parts (<i>Sources</i> : FRIM)	6
Figure 2.4: Transverse section of OPF at low magnification (4x). F = Fiber; P =Parenchyma; Mx = Metaxylem; Px = Protoxylem; Ph = Phloem (Source: Abdul Khalil <i>et al.</i> , 2006).....	8
Figure 2.5: Transverse section of OPF at high magnification (20x). F = Fiber; P =Parenchyma; Mx = Metaxylem; Px = Protoxylem; Ph = Phloem (Source: Abdul Khalil <i>et al.</i> , 2006).....	9
Figure 2.6: Transverse section of multi layered structure of OPF at high magnification (17 000x). ML = Middle lamella; P = Primary wall; S1, S2 & S3 = Secondary wall sub layers. (Source: Abdul Khalil <i>et al.</i> , 2006).....	9
Figure 2.7: The lignin precursors; (I) = p-coumaryl; (II) = coniferyl and (III) = sinapyl alcohol	11
Figure 2.8: Hemicellulose	12
Figure 2.9: Cellulose fibrillous structures : (a) low crystallinity; (b) high crystallinity; (c) folded models (Source : N. Mohd Nor, 2008).....	13
Figure 2.10: The distribution of oil palm plantation hectares in Peninsular Malaysia, Sabah and Sarawak from 1975-2008	16
Figure 2.11: The potential WPT area from years 2011 – 2032	17
Figure 2.12: The area of potential WPT available annually in Peninsular Malaysia, Sabah and Sarawak	17
Figure 2.13: The number of potential WPT available annually in Peninsular Malaysia, Sabah and Sarawak	18
Figure 2.14: Amount of dry matter weight of fronds available annually from potential WPT in Peninsular Malaysia, Sabah and Sarawak	19
Figure 2.15: Schematic representation of effect of pretreatment on biomass (Mosier, 2005).....	20
Figure 3.1: Calibration curve of glucose	25
Figure 4.1: Pareto chart	27
Figure 4.6: Graph of predicted versus actual of glucose conversion.....	29
Figure 4.2: Percentage contribution of factors	29
Figure 4.3: Interaction graph between pH and reaction time	30
Figure 4.4: Interaction graph between enzyme loading and temperature.....	31
Figure 4.5: Interaction graph between agitation speed and enzyme loading	32

LIST OF TABLES

Table 2.1: The area under oil palm plantation (mature and Immature) by states in 2006	4
Table 2.2: The physical parts of the tree that will be acquired during felling with an estimated oven dried weight.....	6
Table 2.3: The type and amount of the biomass and residues produced from these activities and their level of usage	7
Table 2.4: Types of lignocellulosic materials and their current uses.....	14
Table 2.5: The amount of dry matter weight of the fronds that will be generated from the WPT at time of felling	18
Table 3.1: Statistical analysis using Design Expert Software	25
Table 4.1: Contribution of factors	26
Table 4.2: Statistical analysis of the effects of enzyme hydrolysis	27
Table 4.3: Diagnostics case statistic	28

LIST OF ABBREVIATIONS

°C	degree Celsius
g	gram
g/l	gram per liter
g/ml	gram per mililiter
ha	hectare
ml	mililiter
mm	millimeter
nm	nanometer
rpm	rotation per minute
w/w	weight per weight

Greek

α	coefficient of equation
----------	-------------------------

LIST OF ABBREVIATIONS

CPO	crude palm oil
DNS	3-5-Dinitrosalicylic acid
DP	degree of polymerization
EFB	empty fruit bunch
FFB	fresh fruit bunch
FPOPF	fiber pressed oil palm frond
MPOB	Malaysian Palm Oil Board
OD	oven dried
OPEFB	oil palm empty fruit bunch
OPF	oil palm frond
OPT	oil palm tree
POME	palm oil mill effluent
TEM	Transmission Electron Microscopy
WPT	waste palm tree

Chapter 1 Introduction

1.1 Background

The major plantations of commercial crops including rubber, oil palm, cocoa and pineapple are the strength of agricultural production in Malaysia which engages almost all of the arable land. Of the commercial plantation crops, oil palm industry is the important contributor to Malaysian economy with more than 5.00 million hectares of planted areas in 2011; grow by 3% against 4.85 million hectares in 2010. Because of the rapid development of oil palm production in Malaysia, oil palm industry produced highest quantity of biomass for around 80 million dry tones in 2010. The different types of biomass from oil palm industry included empty fruit bunch (EFBs), palm oil mill effluent, fiber, shell, wet shell, palm kernel, fronds and trunks (A. Noraishah., 2012). In 2009, approximately 15.2 and 17.5 million tons (wet weight) of OPT and OPEFB were produced in Malaysia. In spite of this, the most abundant biomass from oil palm plantation is not OPEFB or OPT. The most produced oil palm biomass is oil palm frond (OPF), which amounted to 83 million tons (wet weight) every year and have been found to be a highly favorable source (MPOC, 2010). OPF is obtained in the middle of pruning for harvesting fresh fruit bunch (FFB) that is why it is obtainable daily. OPF is now under-utilized as the plantation owners believe that all the OPF is required for nutrient recycling and soil conservation (Hassan et al., 1994; Wan Zahari et al., 2002). Therefore; pruned fronds are simply left in the plantation. However, the study shows that OPF does not contain high metal contents as widely thought, but contain high carbohydrates in the form of simple sugars. Therefore, part of the OPF can be utilized for other purpose without scarifying the nutrient recycling process (M. Zahari, M. Zakaria, H. Ariffin et al., 2012).

1.2 Problem statement

An excessive amount of oil palm frond (OPF) from oil palm industry will not just caused the improper disposal problem, but also generally caused environmental pollution when the waste are left to go rotten on the grounds or burnt on the plantation sites (K. Lim, Z. Zainal, G. Quadir et al., 2000; N. Mohd Nor., 2008). In spite of this, the large numbers of oil palm fronds (OPF) containing lignocellulosic materials were potentially to be utilized as renewable material. Consumption of all these materials is not going to only fix the sufficient disposal, but as well as contribute side income for farmers and generate more job (O. Akpinor, K. Erdogan, S. Bostanci., 2009; R. Howard, E. Abotsi et al., 2003).

1.3 Research objective

This work aims to study the factors that affect the production of renewable sugar from fiber pressed oil palm frond (FPOPF) through enzyme hydrolysis using Design Expert Software.

1.4 Research scope

The scopes in this research are functioning as a guideline to achieve objective. The scopes are:

- To identify the effect of the pH (4 – 5.6), temperature (35 - 65°C), enzyme concentration (1.5 - 6%), reaction time (3 – 72 hours) and agitation speed (50 – 200 rpm) towards the enzymatic hydrolysis.
- To quantified glucose yield by applying DNS method.
- To analysed the experimental data by using Design Expert Software.

1.5 Rationale and significance

Based on the research scopes mentioned above, the following rationale and significance that we could get have been outlined.

- i. It shall reduce the huge production of biomass residue.
- ii. It shall reduce deforestation and environmental problem.
- iii. Alternative way to produce valuable product from oil palm biomass residue.
- iv. New substitute of raw material for renewable sugar production.
- v. It shall reduce factory's waste disposal costs.
- vi. It shall reduce termites' problem in plantation because of Zero Burning Policy.

1.6 Organization of thesis

A review of literature is presented in Chapter 2. This chapter review about the biomass which is oil palm frond. Chapters 3 describe the experimental work carried out in the thesis where focuses on the alkaline pretreatment and different parameters that affect the enzymatic hydrolysis.

The remaining chapters cover a number of additional investigations carried out as a part of this study:

- Chapter 4 contains result and discussion about the alkaline pretreatment and enzymatic hydrolysis. The results were analysed using Design Expert Software
- Chapter 5 is the conclusion of the study.

Chapter 2 Literature Review

2.1 Lignocellulose Biomass

Lignocellulose is a common term for describing the major components practically in most plants, which are cellulose, hemicelluloses, and lignin. Lignocellulose is a complex matrix, composed of a variety of polysaccharides, phenolic polymers and proteins. Cellulose is the main component of cell walls of land plants. Lignocellulosic biomass comprises of various materials with distinctive physical and chemical characteristics. It is the non-starch based fibrous part of plant material.

First-generation lignocellulose source is primarily from food crops such as grains, sugar beet and oil seeds. Their sustainable production is under scanner, as is the possibility of creating unnecessary competition for land and water used for food and fiber production, thus encroaching on fragile ecosystems like wetlands, forests, and shallow hills.

The cumulative effects of these issues have increased the interest in utilizing non-food biomass and agricultural residues. Feedstock from lignocellulose materials includes corn stover, cereal straw, bagasse, forest residues, and purpose-grown crops such as vegetative grasses and short rotation forests. These second-generation lignocellulosic could prevent many of the concerns facing first-generation lignocellulosic and potentially offer better cost reduction potential in the longer term. Importantly, lignocellulosic feedstock does not interfere with food security (Salman Zafar, 2014). Figure 2.1 shows the first and second generations of lignocellulosic sources.

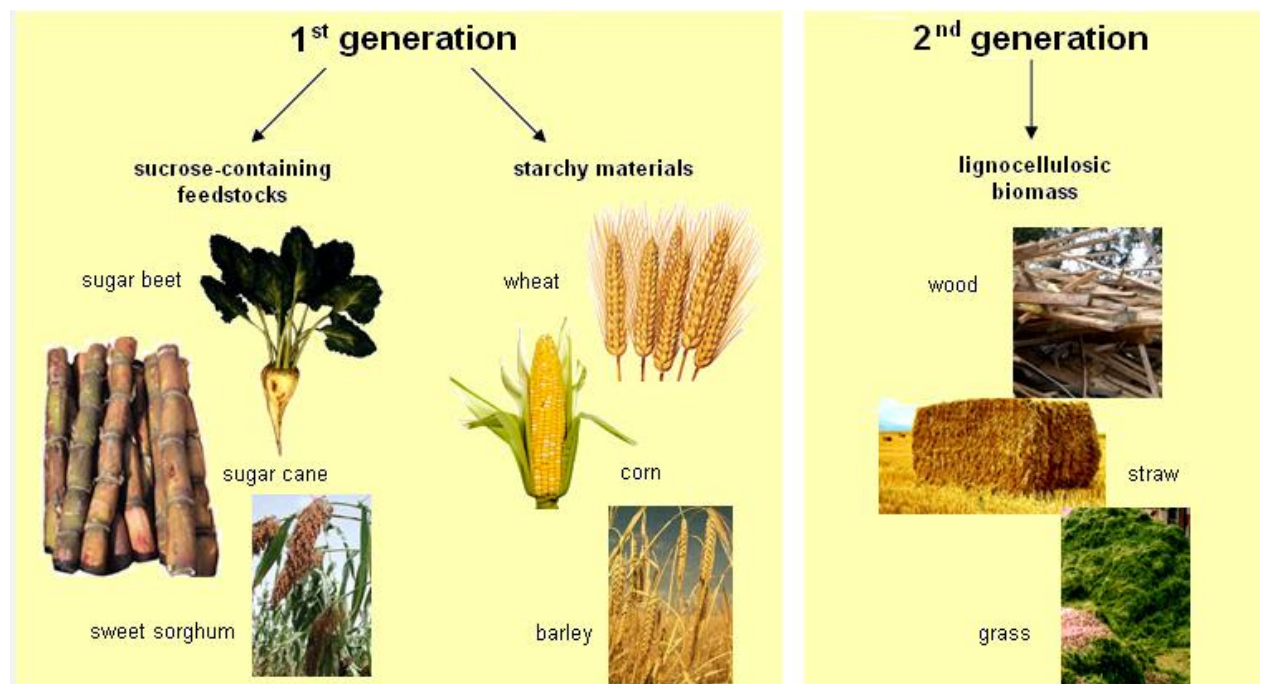


Figure 2.1: The first and second generations of lignocellulosic sources

2.2 Oil Palm

In Malaysia, the most beneficial plant is African oil palm *Elaeis guineensis* which originated from the tropical rain forests of West Africa. It produces palm oil and palm kernel oil, which are commonly used in food as well as other industries such as detergents and cosmetics. In 1871 the oil palm was introduced through the Singapore Botanic Gardens as an ornamental tree. It was commercially exploited as an oil crop (Wan Rosli et al., 2007) only from 1911 when the first oil palm estate was established.

In 1911, the first commercial planting was done at Tenammaran Estate; Kuala Selangor however the progress was initially very slow. It was only during the last 50 years that plantation development was accelerated through large scale investments in the cultivation of the oil palm (Yusof, 2007).

The oil palm tree bears fruit at the age of approximately for a couple of years. The fruit takes roughly five to six months to grow before it is ready for harvest. Its economic life is around 25-30 years, at which stage the tree is felled for replanting. The fruits are developed in large condensed infructescence and are often called fresh fruit bunches (FFB). The size and weight of every bunch varies considerably based on the age and growing conditions. The weight ranges from 8-16 kg per bunch. Table 2.1 shows the area under oil palm plantation (mature and Immature) by states in 2006.

Table 2.1: The area under oil palm plantation (mature and Immature) by states in 2006

State	Mature	Immature	Total
Johor	607,663	63,762	671,425
Kedah	71,705	4,624	76,329
Kelantan	75,825	18,717	94,542
Melaka	49,105	3,127	52,232
N. Sembilan	141,864	19,208	161,072
Pahang	551,713	71,577	623,290
P. Pinang	13,895	224	14,119
Perak	321,656	26,344	348,000
Perlis	258	0	258
Selangor	121,140	7,775	128,815
Terengganu	137,866	26,199	164,065
P. Malaysia	2,092,690	241,557	2,334,247
Sabah	1,139,535	99,962	1,239,497
Sarawak	471,029	120,442	591,471
Sabah/Sarawak	1,610,564	220,404	1,830,968
Malaysia	3,703,254	461,961	4,165,215

Source: MPOB

The fast increase in plantation area in Malaysia implies the economics significance of this plantation crop as well as the increasing world demand for palm oil. Malaysia is the world's largest producer and exporter of palm oil and also currently producing around 50 % of the world's supply of palm oil. Indonesia is the next largest world producer of palm oil producing around 30 % of world palm oil volume. Apart from palm oil, the industry

also produces great quantities of lignocellulosic residues like trunks, fronds and the empty fruit bunches (EFB) with an estimated amount of 30 million tons (N. Mohd Nor, 2008). Figure 2.2 shows the photograph of oil palm tree.



Figure 2.2: The photograph of oil palm tree

2.3 Oil Palm Biomass

Waste palm tree (WPT) at 25 years of age consists of different physical parts (Figure 2.3). Table 2.2 shows the physical parts of the tree that will be acquired during felling with an estimated oven dried weight. The main part by fresh weight is the trunk (70%), followed by rachis (20.5%) and leaflets (6.53%). The moisture contents (depending on O.D. weight) of the various parts vary between 95% and 78%. Since one hectare of an oil palm plantation consists of between 136-140 trees, the sum of dry matter (tons/ha) of the various parts available during felling on a per hectare basis can also be predicted.

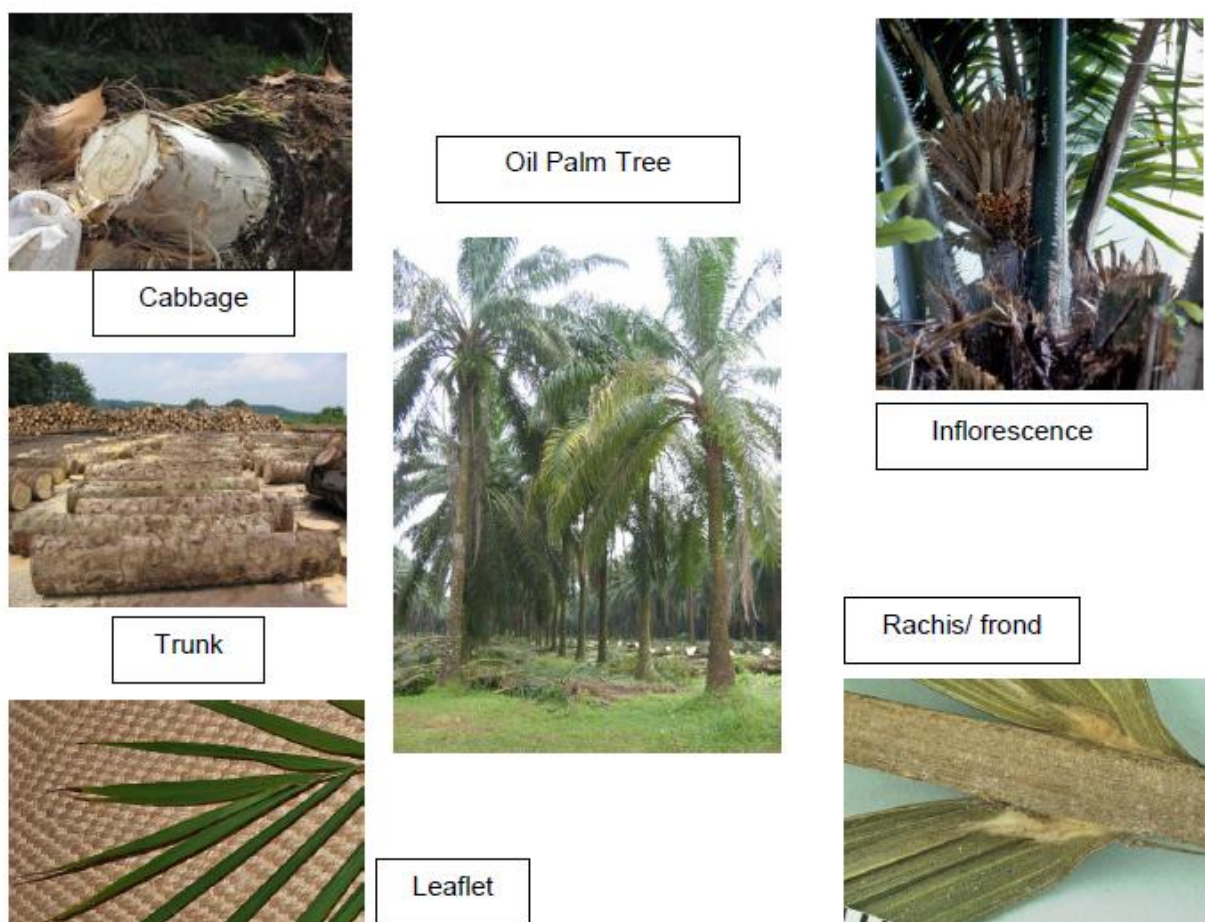


Figure 2.3: WPT at 25 years of age consists of different physical parts (Sources: FRIM)

Table 2.2: The physical parts of the tree that will be acquired during felling with an estimated oven dried weight

WPT component	Average fresh weight (kg)	Weight percentage (%)	Estimated oven dried (OD) weight (kg/free)	Oven dried weight (ton/ha)
Trunk	1507.50	70.0	301.50	41.07
Leaflets	145.00	6.53	58.00	7.69
Rachis	452.50	20.5	117.70	16.00
Spears	42.75	1.92	9.40	1.28
Cabbage	44.50	2.00	4.50	0.60
Inflorescence	134.50	1.11	6.30	17.56
Total weight	2217.50	100.00	497.30	0.86

Source: FRIM

In Malaysia the palm oil industry involves plantation (upstream) and mill (downstream) activities. These activities produce various kinds of residues that are also reported as the residues from the palm oil industries. In 1998 the type and amount of the biomass and residues produced from these activities and their level of usage are demonstrated in Table 2.3. The majority of these biomass and residues are utilized within the system for mulching/fertilizer and for energy production at the mill. From these, the biomass residues generated from replanting activities are just the trunks and fronds at replanting. Pruned fronds are available throughout the year during fruit harvesting.

Table 2.3: The type and amount of the biomass and residues produced from these activities and their level of usage

Biomass	Quantity produced (mil tons)	Quantity utilized (mil tons)	Utilized (%)	Method of utilization
Pruned fronds	27.20	25.83	95	Inter-row mulching in plantations
Trunks and fronds at replanting	1.38	1.10	80	Left to degrade in the fields as mulch to newly planted palms
Mesocarp fibre	3.56	3.20	90	Fuel
Palm kernel shell	2.41	2.17	90	Fuel
Palm oil mill effluent (POME)	1.43	0.50	35	Nutrient source & organic fertilizer
Empty fruit bunch (EFB)	3.38	2.20	65	Left to degrade in the fields as mulch and bunch ash
Crude palm oil (CPO)	39.36	35.00	-	-

Source: FRIM

Even though the main portions of the felled trunks and fronds are claimed being utilized as mulch, there were no reports on the amount actually needed by young palm trees, since fertilizers continue to be being used at the same rate for mulched and un-mulched trees. Mulching was reported as a method of soil surface moisture retention, and is also being done in oil palm plantations by means of cover crops. The other 20% of the WPT is most likely being wasted away when poisoning methods are employed to get rid of old palm trees. WPT is usually used by local communities for temporary structural use for example small bridges and for road maintenance around the village and plantations.

2.3.1 Oil Palm Frond

Oil palm frond has several sizes of vascular bundles which are widely imbedded in thin-walled parenchymatous ground tissue. Every bundle consists of a fibrous sheath, vessels, fibers, phloem and parenchymatous tissues. Xylem and phloem tissues are obviously distinguishable (Abdul Khalil et al., 2006). Phloem is split up into two separate areas in every bundle. Several vascular bundles also have several well-defined protoxylem elements. Protoxylem and metaxylem vessels in the bundle are split up by a layer of parenchyma cells. Within the stem and leaves, proto- and metaxylem vessels are split up by at least one layer of live parenchyma cells which form a living boundary to enable gas bubbles to transfer (Tomlinson et al., 2001). The Transmission Electron Microscopy (TEM) views of transverse sections of OPF are shown in Figure 2.4 and Figure 2.5.

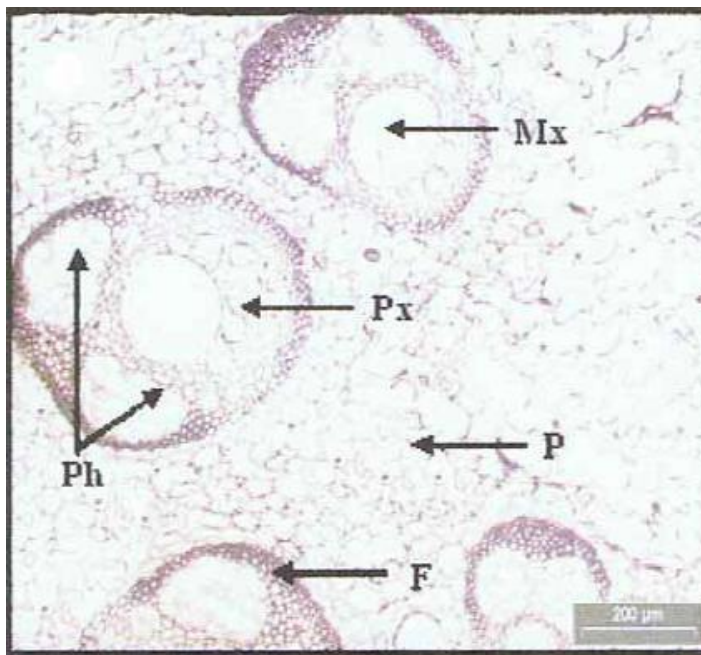


Figure 2.4: Transverse section of OPF at low magnification (4x). F = Fiber; P =Parenchyma; Mx = Metaxylem; Px = Protoxylem; Ph = Phloem (Source: Abdul Khalil *etal.*, 2006)

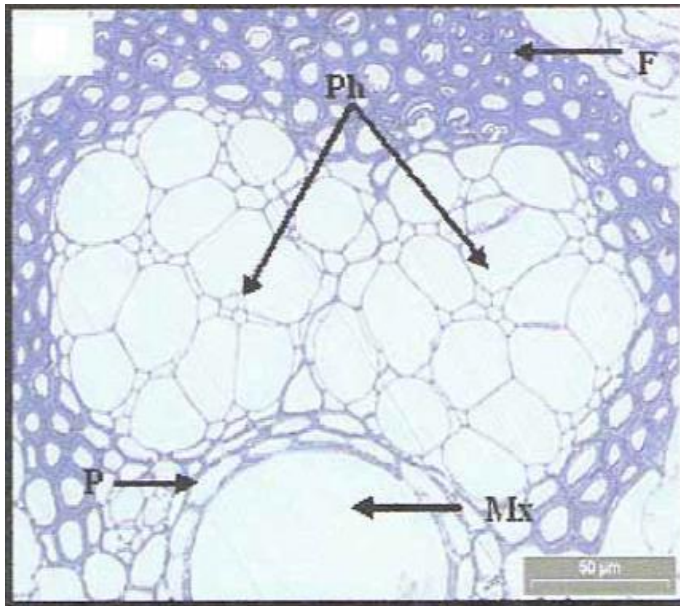


Figure 2.5: Transverse section of OPF at high magnification (20x). F = Fiber; P = Parenchyma; Mx = Metaxylem; Px = Protoxylem; Ph = Phloem (Source: Abdul Khalil *et al.*, 2006)

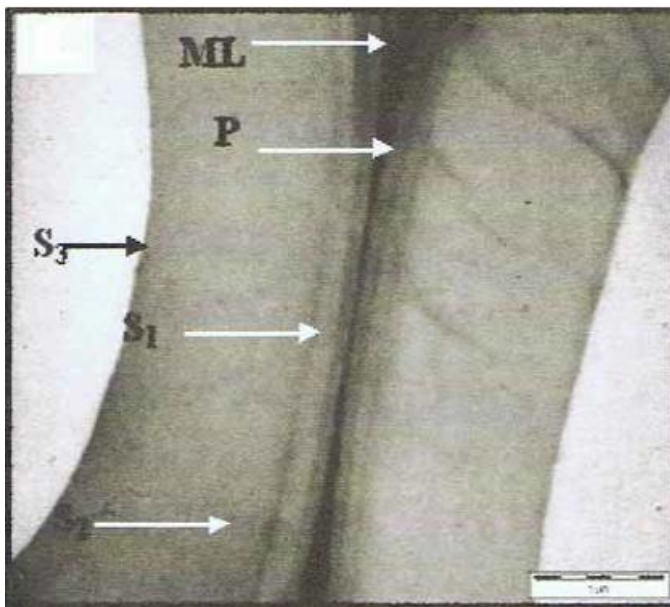


Figure 2.6: Transverse section of multi layered structure of OPF at high magnification (17 000x). ML = Middle lamella; P = Primary wall; S1, S2 & S3 = Secondary wall sub layers. (Source: Abdul Khalil *et al.*, 2006)

The transverse section of multi layered of OPF at high magnification is shown in Figure 2.6. The figure shows that the layered structure of OPF wall has primary (P) and secondary (S1, S2 and S3) wall layer. The primary wall seems as a solid boundary of the cell. The middle lamella indicates an obvious transition to the neighbouring primary wall layers. The S1 layer of OPF is well-defined and could be distinguished from the adjacent S2 layer, since it is the brightest layer than other layers. The existence of distinct S3 layer in the cell wall of OPF also observed using TEM micrograph.

2.3.1.1 Oil Palm Frond Juice

Oil palm frond (OPF) juice contains high sugars content and the OPF are readily available frequently which make it a potential industrial fermentation substrate. Recently, there was a report on the probability of obtaining OPF juice just by pressing the OPF petiole. OPF juice has great amount of sugars, which makes it a potential fermentation feedstock for several beneficial products including polyhydroxyalkanoates (PHA), bioethanol, biobutanol, lactic acid, and succinic acid. Earlier research has proven that OPF juice works well to be utilized as fermentation feedstock as there was no inhibition on microbial growth or product formation, there were no impurities, it was easy to be operated, and it also had no risk on health and safety. OPF juice was proved to be a good fermentation substrate for lab-scale PHA production (M. Zahari, M. Zakaria, H. Ariffin et al., 2012).

2.4 Lignocellulose component in Lignocellulose Biomass

Lignocellulose is the main component of plant cell walls which is basically composed of lignin, cellulose and hemicelluloses. Xylan which consists of 20 – 30 % by weight of wood and agricultural wastes is the main part of the hemicelluloses portion. Xylan therefore represents a preferable resource of renewable biomass that can be applied as a substrate for the making of several beneficial products for instance fuel, solvents and chemicals. However, the industrial usage of such materials has been compromised by a few aspects for example the close association present among the three major parts of the plant cell wall; cellulose, hemicellulose and lignin, as well as the low efficiency by which lignocellulosic substrates are converted via biological processes such as hydrolysis and fermentation.

2.4.1 Lignin

Lignin is a long-chain, heterogeneous polymer comprised mostly of phenyl propane units normally linked by ether bonds and strongly insoluble in water. Lignin is in charge of the structural adhesion of the plant cell wall components because it excludes water when it forms networks by cross-linking along with other saccharide-type molecules in plants. It is presented in every lignocellulosic biomass; hence, any ethanol production process will have lignin as a residue. Lignin is really resistant against chemical, enzymatic and bacterial breakdown, however, just a few organisms, such as rot-fungi as well as some bacteria are able to degrade it. It is an extremely strong component and is most associated with the structural stability of wood. Lignin content in wood can differ from 20 to 35 %. Lignin prevent hydrolysis by protecting cellulose surfaces or by adsorbing and inactivating enzymes. It had been understood that the close union between lignin and cellulose avoided swelling of the fibers, therefore influencing enzyme accessibility to the cellulose. To fix this condition, various research shows that removing lignin magnifies cellulose hydrolysis (Y. Corredor, 2008; N. Mohd Nor, 2008).

The structural units of lignin are different derivatives of phenyl propane with several bonding combinations (Erdtman, 1957). Lignin is made up by oxidative coupling of three main C6 – C3 (phenylpropanoid) units, known as syringyl alcohol, guaiacyl alcohol, and p-coumaryl alcohol, which forms a randomized structure in a tri-dimensional network inside the cell walls. Two major types of lignin units are guaiacyl (with one methoxyl group in phenol ring) and syringyl (with two methoxyl groups).

The structure of lignin is known to differ between cell wall layers and between separate morphological parts of the tree. Commonly inner lamella and outer cell wall layers contain a lot more guaiacyl units compared to the secondary cell wall of hardwoods. The lignin of softwoods is mainly (almost exceptionally) guaiacyl units whereas hardwoods consist of both guaiacyl and syringyl. Apart from the 20 various kinds of bonds found within the lignin alone, lignin is apparently particularly associated with the hemicellulosic polysaccharides. Figure 2.7 shows the lignin precursors; (I) = *p*-coumaryl; (II) = coniferyl and (III) = sinapyl alcohol.

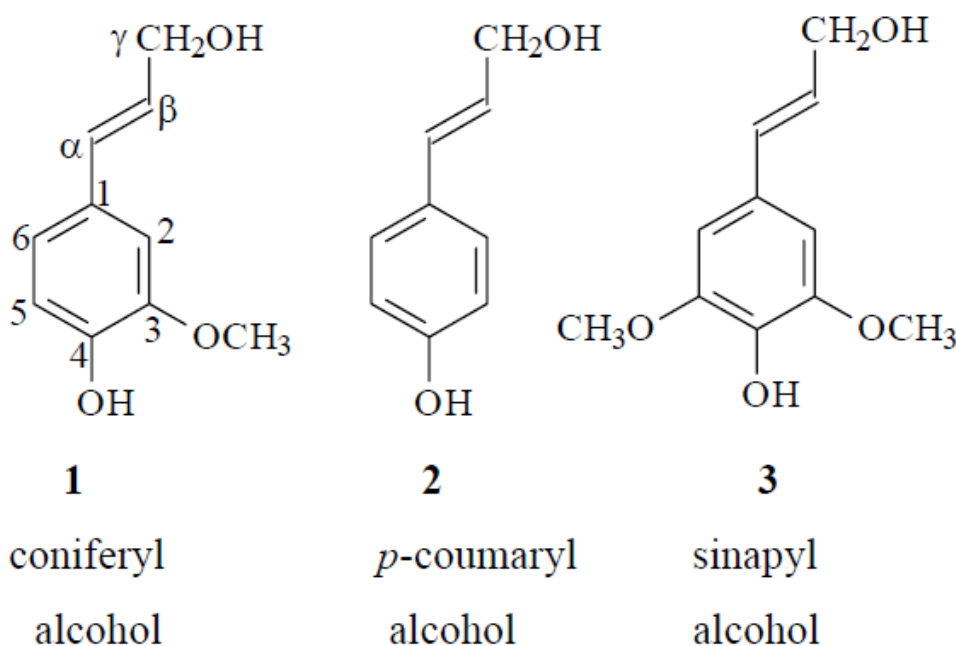


Figure 2.7: The lignin precursors; (I) = *p*-coumaryl; (II) = coniferyl and (III) = sinapyl alcohol

The conversion of cellulose and hemicellulose to fuels and chemicals produces lignin as a by-product. Such by-product can be burned to supply heat and electricity, or utilized to manufacture a variety of polymeric materials. There are several publications on microbial breakdown of lignin; on the other hand, due to high complexity of the problem, an enormous amount of study must be carried out.

2.4.2 Hemicellulose

Hemicelluloses were initially known to be intermediates in the biosynthesis of cellulose. Nowadays it is acknowledged, however, that hemicelluloses are part of a group of heterogeneous polysaccharides which are developed via biosynthetic paths not the same as that of cellulose. Compared to cellulose which is a homopolysaccharide, hemicelluloses are heteropolysaccharides. Hemicelluloses are heterogeneous polymers of pentoses (xylose, arabinose), hexoses (mannose, glucose, galactose), and sugar acids (Figure 2.8). They are usually specified based on the major sugar residue in the backbone, e.g., xylans, mannans, and glucans, with xylans and mannans. Hemicellulose, due to the branched, amorphous character, is relatively easy to hydrolyze (Hamelinck et al., 2005). A few hemicelluloses have mainly xylan, while others have mainly glucomannans (Y. Corredor, 2008).

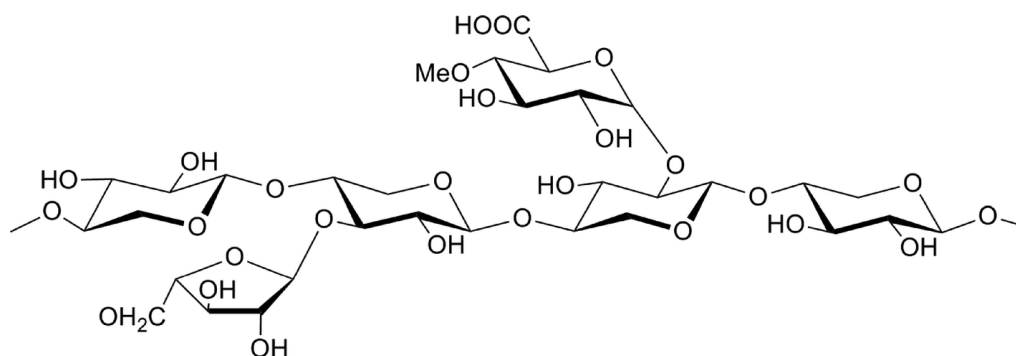


Figure 2.8: Hemicellulose

The hemicellulose content of softwoods and hardwoods vary significantly. Hardwood hemicelluloses are mainly made up of highly acetylated heteroxylans, commonly categorized as 4-O-methylglucuronoxylans. Hexosans are also found however in small quantities as glucomannans. Hardwood xylans are generally labile to acid hydrolysis and may undergo autohydrolysis under relatively moderate situations. On the other hand, softwoods hemicelluloses comprise of glucomannan, galactoglucomannan, arabinan including a few arabino-(4-O methylglucurono)-xylan. Softwood xylans are free from acetyl groups, there are split up as a gel from acidic solutions, one of them, in methylated form, being presented as a robust film, indicating the xylan's linear structure. Due to this fact, softwood hemicelluloses (mainly hexosans) are very proofed against acid hydrolysis rather than hardwood hemicelluloses (mainly pentosans). The average amount of hemicelluloses in wood is 25 – 35 %. Hardwood species contain in average 1.5 times extra hemicelluloses compared to softwood species (N. Mohd Nor, 2008).

In contrast to cellulose, hemicelluloses have lower DP in a range of 50 to 300 with side groups on the chain molecule and are basically amorphous which have a lot more branches and are less crystalline than cellulose. They are easily hydrolysable mainly to xylose when it comes to hardwood and to mannose when it comes to softwood.

2.4.3 Cellulose

In nature, the most numerous organic compound is cellulose which consist more than 50% of all the carbon in vegetation. Regardless of the source, cellulose is believed to be similar in chemical composition, it is insoluble in water and aqueous solutions in alkalis.

Cellulose is a linear homopolymer that contains glucose (D-glucose) units linked by β -(1-4) glycosidic bonds. Usually, the size of cellulose molecule is depends on degree of polymerization (DP). Cellulose chains in primary plant cell walls have DP of in the range of 5,000 to 7,500 glucose monomer units. Cellulose from wood has DP around 10,000, while cotton is around 15,000.

Cellulose molecules are completely linear with a strong probability to form intra and intermolecular hydrogen bonds. Long molecules of cellulose from micro fibrils build the structure of a cell wall (fiber wall) where highly organized (crystalline) parts alternate with less organized (amorphous) parts. The crystalline part wherein the linear molecules of cellulose are bonded laterally by hydrogen bonds is distinguished by the cellulose lattice which broadens over the whole cross-section of the micro-fibrils. This crystalline part is bordered by a layer of cellulose molecules that show several degrees of parallelism.

The less organized part is known as the paracrystalline or amorphous part. The disorganized part enables disintegration of the cellulose by hydrolysis into rod-like particles with aqueous, non-swelling, strong acid (Y. Corredor, 2008). These structures make cellulose a rigid, strong, dense, partly crystalline, chemically and enzymatically resistant features.

Yet somehow, a number of cellulose which is roughly 10 % can also be found in an amorphous state. The cellulose fibrillous structure is shown in Figure 2.9.

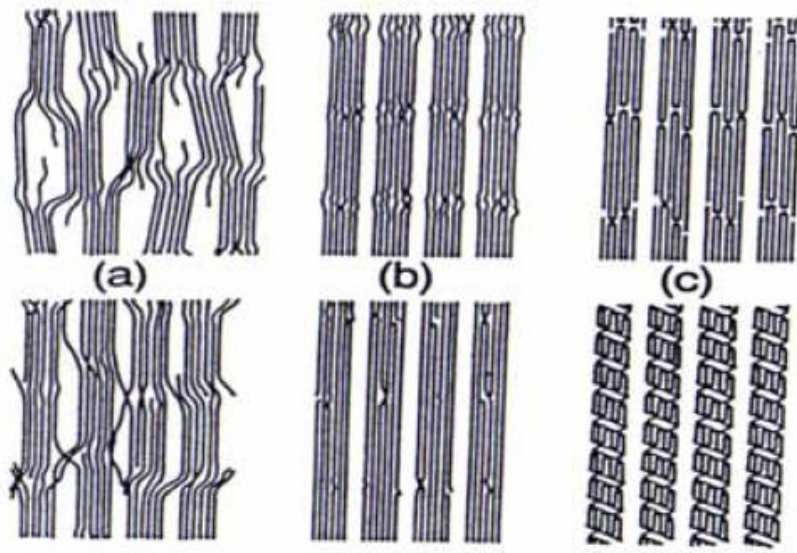


Figure 2.9: Cellulose fibrillous structures: (a) low crystallinity; (b) high crystallinity; (c) folded models (Source: N. Mohd Nor, 2008)

The crystalline structure of cellulose can be destroyed and turned into amorphous form under over 250°C of water or strong acid. The enzymatic attack needs particular pretreatment methods, or else the saccharification yields are dramatically low (N. Mohd Nor, 2008).

2.5 Application of Lignocellulose Component

Biomass is generally defined as the mass of organic material from any existing biological material, and by extension, any large mass of biological matter. Numerous types of biomass resources can be found (Table 2.4) on earth for conversion into bioproducts. These may involve entire plants, plant parts, plant constituents, processing byproducts, materials of marine origin and animal byproducts, municipal and industrial wastes (Smith et al., 1987). These resources are useful to generate new biomaterials and this will need a passionate knowledge of the composition of the raw material whether it is entire plant or constituents, to ensure that the required functional elements is available for bioproduct production.

Table 2.4: Types of lignocellulosic materials and their current uses

Lignocellulosic material	Residues	Competing use
<i>Grain harvesting</i> Wheat, rice, oats barley and corn	Straw, cobs, stalks, husks	Animal feed, burnt as fuel, compost, soil conditioner
<i>Processed grains</i> Corn, wheat, rice, soybean	Waste water, bran	Animal feed
Fruit and vegetable harvesting	Seeds, peels, husks, stones, rejected whole fruit and juice	Animal and fish feed, some seeds for oil extraction
Fruit and vegetable processing	Seeds, peels, waste water, husks, shells, stones, rejected whole fruit and juice	Animal and fish feed, some seeds for oil extraction
Sugarcane other sugar products	Bagasse	Burnt as fuel
<i>Oils and oilseed plants</i> Nuts, cotton seeds, olives, soybean etc.	Shells, husks, lint, fibre, sludge, press cake, wastewater	Animal feed, fertilizer, burnt fuel
Animal waste	Manure, other waste	Soil conditioners
<i>Forestry-paper and pulp</i> Harvesting of logs	Wood residuals, barks, leaves etc.	Soil conditioners, burnt
Saw and plywood waste	Woodchips, wood shavings, saw dust	Pulp and paper industries, chip and fiber board
Pulp and paper mills	Fiber waste, sulphite liquor	Reused in pulp and board industry as fuel
Lignocellulose waste from communities	Old newspapers, paper, cardboard, old boards, disused furniture	Small percentage recycled, others burnt
Grass	Unutilized grass	Burnt

Source: Smith et al., 1987

2.5.1 Chemicals

Bioconversion of lignocellulosic wastes could make an important contribution to the production of organic chemicals. More than 75% of organic chemicals are produced from five primary base-chemicals which are ethylene, propylene, benzene, toluene and xylene that utilized to synthesis other organic compounds. These organic compounds often are utilized to produce a variety of chemical products such as polymers and resins. The aromatic compounds might be produced from lignin while the low molecular mass aliphatic compounds can be derived from ethanol produced by fermentation of sugar generated from the cellulose and hemicellulose.

2.5.2 Bio-fuel

The demand for ethanol has the most important market where ethanol is either utilized as a chemical feedstock or as an octane enhancer or petrol additive. The production of ethanol from sugars or starch effects unfavourably on the economics of the process, hence making ethanol more costly rather than fossil fuels. This is why the technology development focus for the production of ethanol has shifted to the utilization of residual lignocellulosic materials to reduce production costs.

2.5.3 Other high-value bioproducts

Nowadays a lot of products including organic acids, amino acids, vitamins and a variety of bacterial and fungal polysaccharides like xanthan are made by fermentation using glucose as the basic substrate. However in theory these same products could be produced from “lignocellulose waste”. There are possible value added products that can be derived from lignin. Vanillin and gallic acid are the two most regularly discussed monomeric possible products which have attracted interest. Vanillin can be used for different applications like being an intermediate in the chemical and pharmaceutical industries for the production of herbicides, anti-foaming agents or drugs such as papaverine, L-dopa as well as anti-microbial agent, trimethoprim. Additionally it is applied in household products including air-fresheners and floor polishes.

Hemicelluloses are of particular industrial attraction because these are easily available bulk source of xylose from which xylitol and furfural can be derived. Xylitol utilized rather than sucrose in food as a sweetener, has odontological purposes for example teeth hardening, remineralisation, and as an antimicrobial agent, it is applied in chewing gum and toothpaste formulations. The yield of xylans as xylitol by chemical signifies is just around 50-60% making xylitol production pricey. A variety of bioconversion methods are studied for the production of xylitol from hemicellulose using microorganisms or their enzymes. Furfural is employed in the production of furfuralphenol plastics, varnishes and pesticides.